Enzyme Linked Immunosorbent Assay for Fumonisin B1 Detection in Local Corn Seeds from Baghdad-Iraq

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Abstract
Fungi produce a series of toxic compounds on corn, especially Fumonisin B1 (FB1) toxin produced by Fusarium spp. and promoting cancer activity in humans and animals. This study aimed to isolate and identify fungi associated with local corn seeds and the detection for the presence of FB1 by using ELISA technique. Thirty samples of corn ears were collected from silos and markets in Baghdad city during the period from November 2018 to March 2019. The present study found that Fusarium was the dominant isolate among fungi in terms of the relative density 57.07%, followed by Aspergillus 31.17%, Rhizopus 3.36%, Alternaria 2.88%, Mucor 2.16%, Penicillium 1.92%, Trichothecium 0.96%, and Helminthosporium 0.48%. FB1 was detected in all samples of the silos and markets with a concentration range of 13.69 - 175.54 µg/kg. There were no significant differences in FB1 concentration among samples collected from the silos and markets. Also, no relationship was found between the number of infected seeds by Fusarium spp. and FB1 concentrations.

Keyword: FB1, Fusarium, ELISA, Corn

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الخلاصة
تنتج الفطخيات سلسلة من المركبات السامة على حبوب الذرة خصوصا السم فيومونيسين B1 الذي ينتج من قبل Fusarium وهو سم محفز للسرطان عند الإنسان والحيوان. هدفت الدراسة إلى عزل وتشخيص الفطخيات المصاحبة لحبوب الذرة المحلية والتحري عن وجود السم فيومونيسين B1 باستخدام تقنية الإمارات. تم جمع ثلاثين عينة من عرابيذ الذرة المحلية من مخازن الحبوب والأسواق المحلية في محافظة بغداد خالل الفترة تشرين الثاني 2018 إلى اذار 2019. وأظهرت النتائج أن Fusarium كان من العرابيذ السائدة 13.69-175.54 ميكروغرام / كغم. لا يوجد فرق معنوي في تراكم السم فيومونيسين B1 بين المخازن.

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Introduction

Different filamentous fungi are able to produce toxic compounds with small molecular weight, called mycotoxin [1]. *Fusarium*, *Aspergillus*, and *Penicillium* are the main toxigenic genera that are able to invade grains in the field and during storage period under suitable conditions of temperature and moisture [2]. Corn (*Zea mays* L.) is a worldwide staple food crop which can be invaded by certain fungi, especially *Fusarium* species that produce diverse toxins such as Fumonisin B1 [3]. FB1 is present mainly in corn and in other grains, such as oat, rice, and wheat [4]. Previous studies found that the consumption of FB1 has been related to human and animal diseases, with hepatotoxic, neurotoxic, teratogenic, nephrotoxic, immunosuppressive, carcinogenic, and pulmonary effects [5, 7]. Enzyme Linked Immuno-Sorbent Assay (ELISA) technique is the most popular method used for the detection of mycotoxin in food and feed [8]. There are several advantages to ELISA over other techniques; it is simple, low-cost, sensitive, and specific. It requires a small sample size and can analyze a large number of samples at the same time [9]. Therefore, this study aimed to the isolation and identification of fungi along with the direct detection of the presence of FB1 in corn ears by using ELISA.

Materials and Methods

1. Samples collection

Thirty samples of corn ears were collected from local markets and silos of Baghdad province (fifteen sample from silos and fifteen from markets), during the period from November 2018 to March 2019.

2. Fungal Isolation and Identification

Isolation of fungi from grains was performed on 50 seeds from each corn sample. The surfaces of seeds were sterilized by 2% sodium hypochlorite solution for one minute and washed by sterilized D.W. three times. The grains were left for drying by sterilized filter papers, then 10 grains were seeded on potato dextrose agar (PDA: an enrichment medium for the isolation of all fungi) and malachite green agar 2.5 (MGA 2.5: a selective medium for the isolation of *Fusarium* spp.) media containing chloramphenicol (50 mg/L). The plates were incubated for seven days at 25°C. All fungal isolates were identified to species level by using Scotch tape preparation and slide culture technique, depending on macroscopic and microscopic appearance [10]. The values of relative density (RD), isolation frequency (IF), and incidence (IN) of infected seeds per sample were calculated according to Jedidi *et al.* [11] and Tsedaley *et al.* [12].

\[
RD\% = \frac{\text{No. of isolated fungi species}}{\text{Total No. of fungi}} \times 100
\]

\[
IF\% = \frac{\text{No. of samples occurrence of fungi species}}{\text{Total No. of samples}} \times 100
\]

\[
IN\% = \frac{\text{No. infected seeds}}{\text{Total No. of seeds}} \times 100
\]

3. Detection of FB1 by ELISA

Preparation of samples was performed according to the manufacturer’s instructions, as in the following: 5 gm of grinded maize was added to 25 ml of 70 % methanol in a container and the mixture was shaken for 20 minutes. The sample was centrifuged at 4,000 rpm/10 minutes. One ml of the supernatant was diluted with 1 ml of D.W., then 50 μl of the diluted supernatant per well was used in the experiment. Detection of FB1 by competitive ELISA technique was performed using BiooScientific ELISA kit. The plate was measured optically after adding stop buffer using plate reader with 450 nm wavelength.
Results and Discussion

Thirty samples were analyzed for the presence of Fumonisn B1 and mycoflora in corn ears. Figure 1 shows isolation frequency and relative density values of fungi isolated from all samples. The relative density results showed that Fusarium was the dominant isolate among the other fungi (57.07%), followed by Aspergillus (31.17%), Rhizopus (3.36%), Alternaria (2.88%), Mucor (2.16%), Penicillium (1.92%), Trichothecium (0.96%) and Helminthosporium (0.48%). Whereas, isolation frequency results demonstrated that Aspergillus was the dominant isolate (59.38%), followed by Fusarium (43.75%). In silo samples, the relative density of Fusarium spp. (66.17%) was higher than that recorded in the samples from local markets (41.06%), as shown in Figure 2. The present research identified two main fungus genera (Fusarium and Aspergillus) that are associated with silo and markets’ corn seeds. This result agrees with those of previous studies [2, 12] which reported that Fusarium and Aspergillus are the main genera encountered on corn seeds in tropical areas. Corn seeds could be infected by these two genera during field or storage periods [12]. Also, when corn become contaminated with Fusarium spp. during pre and post-harvest, this leads to an increase in FB1 content; therefore, some studies considered that Fusarium spp. is a field and storage organism [13-15].

![Figure 1](image_url)

**Figure 1**-Isolation frequency and relative density of fungi isolated from corn samples of silo and markets in Baghdad city.
The results revealed that FB1 concentration was 175.54 µg/kg and 172.88 µg/kg in silo and market samples, respectively, as shown in Table (1). There were non-significant differences in the mean of FB1 concentrations between silo and market samples. This finding agrees with that of a previous study which considered Fusarium spp. as a field fungus [16]. Also, the present study found no relationship between the number of infected seeds by Fusarium spp. and FB1 concentration, as shown in Figure 3. Fumonisins production by Fusarium spp. is dependent on fum gene cluster (fum1 - fum9), and the responsible gene for FB1 biosynthesis is fum1[17]. In addition, FB1 production is Fusarium species-dependent and the main producers of FB1 are F. verticillioides and F. proliferatum.

**Table 1- Concentration of Fumonisin B1 in corn samples collected from silo and markets**

<table>
<thead>
<tr>
<th>Location</th>
<th>Number</th>
<th>Mean (µg/kg)</th>
<th>Standard Error</th>
<th>Min.</th>
<th>Max.</th>
<th>ANOVA p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silo</td>
<td>15</td>
<td>112.05</td>
<td>11.71</td>
<td>24.43</td>
<td>175.54</td>
<td>0.753 (NS)*</td>
</tr>
<tr>
<td>Markets</td>
<td>15</td>
<td>117.70</td>
<td>13.37</td>
<td>13.69</td>
<td>172.88</td>
<td></td>
</tr>
</tbody>
</table>

*NS: Not significant \((p < 0.05)\)

**Figure 2**-Relative density of fungi genera isolated from corn samples of silo and markets in Baghdad city.

**Figure 3**-Relationship between number of infected seeds by Fusarium spp. and Fumonisin B1 concentration
The concentration of FB1 in the present study was low when compared with previous studies in Iraq [20]. This may be due to the use of new strategies in plant disease control. Also, this comparison showed that the production of FB1 in corn was higher than that in other plants (rice and wheat) in Iraq and other countries, as presented in Table 3. This indicates that corn and its products are the main contaminated foods by FB1 [4]. Corn became more susceptible to toxigenic fungi that produce FB1, due to many reasons such as harvesting methods, drying process, and methods of transport to other countries, which could cause mechanical damage to the corn. In addition, storage conditions, including high humidity, temperature, insects, rodents, and long period of storage, can damage the grains rapidly and elevate corn mycobiota during storage; therefore, stored and broken maize are more vulnerable than freshly harvested maize [21, 22]. Subsequently, ingestion of contaminated grains by FB1 can affect human and animal health, causing hepatotoxic and nephrotoxic effects [4, 5, 23].

### Table 3-A comparison of Fumonisin B1 concentration between the present and previous studies in Iraq and other countries

<table>
<thead>
<tr>
<th>Ref.</th>
<th>FB1 Concentration (µg/kg)</th>
<th>Plant</th>
<th>Detection method</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>13.69 - 175.54</td>
<td>Corn</td>
<td>ELISA</td>
<td>Iraq</td>
</tr>
<tr>
<td>Al-Zobaidy [24]</td>
<td>0</td>
<td>Rice</td>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td>Hamad &amp; Aki [20]</td>
<td>467.19 - 570.0</td>
<td>Corn</td>
<td>HPLC</td>
<td></td>
</tr>
<tr>
<td>Hammood &amp; Al-Nazzal [25]</td>
<td>0.103 - 2.240</td>
<td>wheat</td>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td>Shephard et al [26]</td>
<td>10-3980</td>
<td>Corn</td>
<td>HPLC</td>
<td>Iran</td>
</tr>
<tr>
<td>Alkadi et al [27]</td>
<td>5-6</td>
<td>wheat</td>
<td>HPLC</td>
<td>Syria</td>
</tr>
<tr>
<td>El-Sayed et al [28]</td>
<td>10-780</td>
<td>Corn</td>
<td>HPLC</td>
<td>Egypt</td>
</tr>
<tr>
<td>Omurtag [29]</td>
<td>0.30-0.32</td>
<td>Corn</td>
<td>HPLC</td>
<td>Turkey</td>
</tr>
<tr>
<td>Altiparmak &amp; Tunali [13]</td>
<td>0.11 - 8.48</td>
<td>Corn</td>
<td>HPLC</td>
<td></td>
</tr>
<tr>
<td>Katta et al [30]</td>
<td>0.1 - 3.5</td>
<td>Corn</td>
<td>HPLC</td>
<td>Washington</td>
</tr>
<tr>
<td>Liu et al [31]</td>
<td>0.2 - 3.0</td>
<td>Corn</td>
<td>HPLC</td>
<td>Taiwan</td>
</tr>
<tr>
<td>Munawar et al [32]</td>
<td>&gt; 0.25</td>
<td>Corn</td>
<td>ELISA</td>
<td>Indonesian</td>
</tr>
</tbody>
</table>

### Conclusions

The present study found no significant differences in FB1 concentrations between corn samples collected from silos and markets. Also, there is no relationship between number of infected seeds by Fusarium spp. and FB1 concentration.

### Acknowledgment

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### References


